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GATA3 abnormalities and the phenotypic spectrum of HDR syndrome

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Abstract

We report on GATA3 analysis and the phenotypic spectrum in nine Japanese families with the HDR syndrome (hypoparathyroidism, sensorineural deafness, and renal dysplasia) (MIM 146255). Fluorescence in situ hybridisation and microsatellite analyses showed heterozygous gross deletions including GATA3 in four families. Sequence analysis showed heterozygous novel mutations in three families: a missense mutation within the first zinc finger domain at exon 4 (T823A, W275R), an unusual mutation at exon 4 (900insAA plus 901insCCT or C901AACCT) resulting in a premature stop at codon 357 with loss of the second zinc finger domain, and a nonsense mutation at exon 6 (C1099T, R367X). No GATA3 abnormalities were identified in the remaining two families. The triad of HDR syndrome was variably manifested by patients with GATA3 abnormalities. The results suggest that HDR syndrome is primarily caused by GATA3 haploinsufficiency and is associated with a wide phenotypic spectrum.

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Keywords: GATA3; HDR syndrome; phenotypic spectrum; mutation analysis

The unique dominantly inherited condition consisting of hypoparathyroidism, sensorineural deafness, and renal dysplasia has been recognised as a distinct clinical entity since the report by Bilous *et al*¹ (MIM 146255). Hasegawa *et al*² found this condition, which they named with the acronym HDR syndrome, in a Japanese girl with a de novo 10p deletion, suggesting that the gene for this syndrome resides on 10p. Subsequently, molecular deletion analyses have been carried out in several patients with features indicative of HDR syndrome, defining the critical region between D10S189 and D10S226 on 10p14–15, distal to the DiGeorge critical region II.^{3,4} In this context, GATA3, a transcription factor with two transactivating domains (TA1 and TA2) and two zinc finger domains (ZF1 and ZF2),^{5,6} has been mapped to distal 10p,⁷ and human GATA3 expression has been detected in the developing parathyroid glands, inner ears, and kidneys, together with the thymus and central nervous system (CNS).^{8,9} Van Esch *et al*¹⁰ performed a positional candidate gene approach, successfully identifying heterozygous GATA3 abnormalities (one nonsense mutation, two

intragenic deletions, and two whole gene deletions) in five families with HDR syndrome.

However, GATA3 haploinsufficiency has been reported in only five families with a fairly typical HDR phenotype,¹⁰ so that the frequency of GATA3 haploinsufficiency and the phenotypic spectrum remain to be determined. Here, we report on further GATA3 analysis in patients with HDR syndrome and discuss clinical features of this syndrome.

Materials and methods

SUBJECTS

This study consisted of nine Japanese families (fig 1). In each family, the proband was ascertained because of having the triad of HDR syndrome. Families C, D, and F have been reported elsewhere.^{2,11,12} For convenience, each person is indicated by a family/pedigree number, for example, the proband of family A is described as case A/II.1.

The HDR triad features of each patient are summarised in table 1. Hypocalcaemia and/or hypoparathyroidism was indicated in 11 of 13 biochemically examined cases. One case (A/II.1) had asymptomatic neonatal hypocalcaemia and repeated blood samplings after the neonatal period did not detect hypocalcaemia but indicated low serum intact parathyroid hormone (PTH) levels. The remaining 10 cases had afebrile convulsions, irritability, or tetany between 1 month and 72 years of age, and were diagnosed as having hypoparathyroidism with definitely or relatively low intact PTH levels; their serum calcium levels were normalised with 1 α (OH) vitamin D therapy. In addition, one case (F/III.1) had a low normal intact PTH level, although there was no discernible hypocalcaemia. Sensorineural deafness was present in nine of 11 cases examined by auditory brainstem response or audiometry. The degree of hearing loss was variable among patients, ranging from mild to profound impairment, and was symmetrical in all cases except for one (F/I.1). In addition, hearing impairment was probable in two cases using hearing aids, and was clinically suspected in two cases with delayed verbal development. Renal lesions were radiologically confirmed or clinically suspected in 13 of 16 cases. Renal aplasia or hypoplasia was shown in five cases by ultrasound or CT scans, pelvicalyceal deformity was found in three cases by intravenous pyelography, vesicoureteral reflux was shown in two cases by voiding cystourethrography, and gross scar was detected in one case by renal scintigraphy. The urinary anomalies were asymmetrical in all but two cases (A/II.1 and

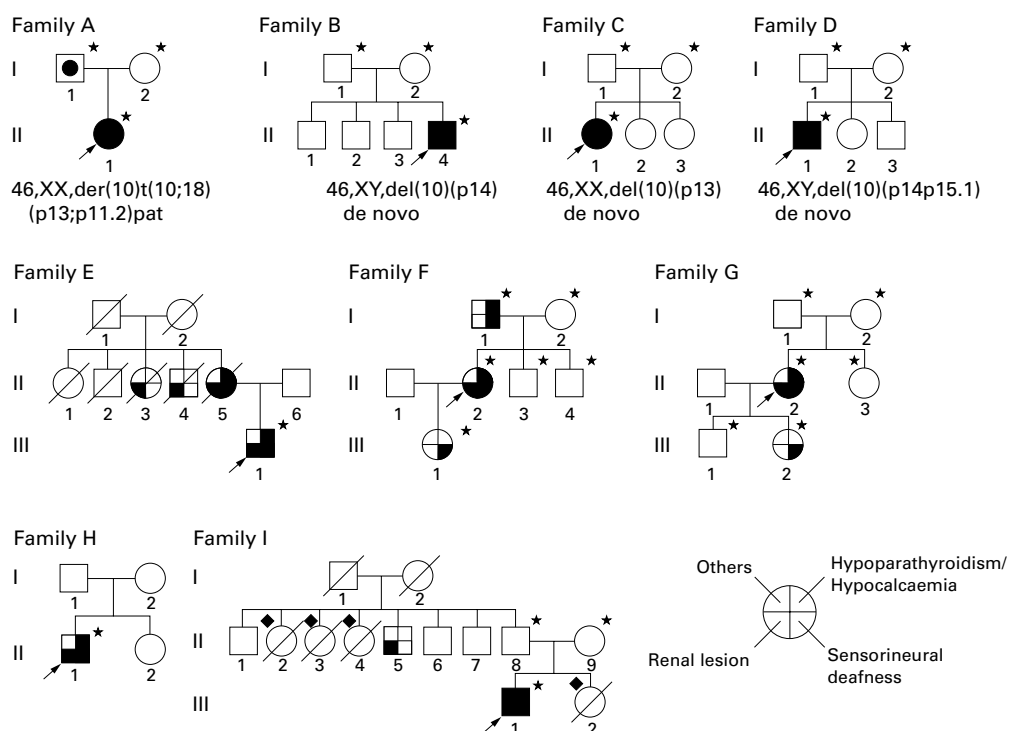


Figure 1 Families A-I analysed in the present study. The proband in each family is indicated by an arrow. The karyotypes of the probands in families A-D are described; the father of family A has a reciprocal 10p;18p translocation. The presence of the HDR triad and other features is indicated by blackening of each quartered area. The asterisks indicate subjects who underwent blood sampling. The black diamonds in family I represent subjects who died in the early neonatal period.

D/II.1). Six cases developed chronic renal failure, and five of them were placed on dialysis. One case (G/II.2) had proteinuria and haematuria suggestive of renal dysplasia.

Several patients had additional features other than the triad of HDR syndrome (fig 1). Case B/II.4 had pyloric stenosis and underwent Ramstedt pyloromyotomy at 1 month of age. Case C/II.1 had a ventricular septal defect, which closed spontaneously. Case D/II.1 experienced cerebral infarction four times between 7 and 20 months of age. Case I/III.1 had bilateral retinitis pigmentosa and severe short stature (-4.4 SD), which were absent in other family members. Cases I/II.2, I/II.3, I/II.4, and I/III.2 died of unknown cause in the neonatal period. In addition, the probands of families A-D manifested various degrees of growth failure, mental retardation, and multiple congenital minor anomalies. By contrast, immune related disorders were absent in all the patients, and immunological studies showed no definite abnormalities in cases A/II.1, B/II.4, and C/II.1 (table 2).

After obtaining appropriate consent, blood samples were taken from 12 affected and 16 clinically normal subjects from families A-I indicated by the asterisks in fig 1.

CYTOMETRIC STUDIES

Chromosome analysis was performed on 50 lymphocytes with standard G banding. For the probands, high resolution G banding was also carried out with ethidium bromide.

DELETION ANALYSIS OF DISTAL 10p

The distal 10p region was examined for deletions including GATA3 by fluorescence in situ hybridisation (FISH) and microsatellite analyses. For FISH analysis, lymphocyte metaphase spreads were hybridised with seven probes defining the loci/region including GATA3 at distal 10p (table 3), together with a 10q telomere probe (2136c3/cosmid)¹⁶ used as an internal signal control. The 554F11/BAC probe was shown to contain all the six exons of GATA3 by polymerase chain reaction (PCR) analysis (data not shown). The 10q telomere probe was labelled with biotin and detected by avidin conjugated to fluorescein, and the remaining probes were labelled with digoxigenin and detected by rhodamine anti-digoxigenin. For microsatellite analysis, leucocyte genomic DNA was amplified by PCR with fluorescently labelled forward primers and unlabelled reverse primers defining 12 loci on distal 10p (table 3), and the PCR products were determined for the fragment size on ABI PRISM 310 autosequencer using GeneScan software (Applied Biosystems, Foster City, USA). The resources of FISH probes and microsatellite primers are shown in the legend to table 3. The genetic distance of the loci examined from the 10p telomere is described in table 3, and the 10p genetic map is shown in fig 2A.

MUTATIONAL ANALYSIS OF GATA3

The GATA3 gene was examined for mutations by direct sequencing for both strands on the autosequencer. In brief, leucocyte genomic

Table 1 Summary of patients examined in the present study

Patients	Hypoparathyroidism/hypocalcaemia				Sensorineural deafness		Renal lesion			
	Case	Age* (y)	Ca ²⁺ (mmol/l)	P ⁴⁺ (nmol/l)	Intact PTH ¹⁻³⁴ (ng/l)	Assessment (age at Dx)	Hearing level‡ (dB)	Assessment (age at Dx)	Findings	Assessment (age at Dx)
A/II.1	Neonatal	1.85	NE	NE	NE	Transient (6 d)	>105 (B) on ABR	Yes (5 mth)	Pelviclyceal deformity (B)	Yes (13 mth)
	1.5	2.40	2.00	6.0	6.0					
	3	1.45	3.51	Undetected	Undetected	Yes (1 mth)	>90 (B) on ABR	Yes (3 y)	Gross renal scar (L)	Yes (3 y)
B/II.4										
C/II.1	9	1.25	2.55	18.0	18.0	Yes (2.5 y)	90–100 (B) on ABR	Yes (2.7 y)	VUR grade 4 (R) and 5 (L)	Yes (2.7 y)
D/II.1	14	1.85	2.13	Undetected	Undetected	Yes (1.4 y)	40–50 (B) on AM	Yes (7 y)	Pelviclyceal deformity (L)	Yes (7 y)
E/III.1	46	1.20	2.07	Undetected	Undetected	Yes (39 y)	70 (B) on AM	Yes (childhood)	Renal hypoplasia (L)	Yes (36 y)
II.3	(80)§	NE	NE	NE	NE	Uncertain	NE	Uncertain	CRF, on dialysis	Yes (50s)
II.4	(85)§	NE	NE	NE	NE	Uncertain	NE	Uncertain	CRF, on dialysis	Yes (70s)
II.5	(80)§	1.75	1.81	15.0	15.0	Yes (72 y)	NE	Probable**	Renal hypoplasia (L)	Yes (67 y)
F/II.2	27	1.45	2.16	Undetected	Undetected	Yes (25 y)	40–50 (B) on AM	(childhood)	CRF, on dialysis	Yes (27 y)
I.1	57	1.75	1.81	2.0	2.0	Yes (57 y)	60–70 (R) and >110 (L) on AM	Yes (27 y) Yes (childhood)	Renal aplasia (R)	No
III.1	2	2.55	1.68	14.0	14.0	No	Normal on ABR	Possible††	Normal on ultrasound and urine analysis	No
G/II.2	33	2.03¶	1.13¶	Undetected	Undetected	Yes (3 y)	NE	Probable**	Proteinuria & haematuria	Probable (20s)
III.2	3	2.20	1.74	20.0	20.0	No	Normal on ABR	(childhood)	Normal on ultrasound and urine analysis	No
H/II.1	19	1.13	3.16	Undetected	Undetected	Yes (14 y)	30–40 (B) on AM	Possible††	Renal aplasia (R)	Yes (14 y)
I/III.1	10	1.15	2.78	Undetected	Undetected	Yes (1 mth)	>105 (B) on ABR	Yes (10 y)	CRF	Yes (7 y)
II.5	60	NE	NE	NE	NE	Uncertain	NE	Uncertain	CRF, on dialysis	Yes (50s)

R, right; L, left; B, bilateral; NE, not examined; PTH, parathyroid hormone; Dx, diagnosis; dB, decibels; ABR, auditory brainstem response; AM, audiometry; VUR, vesicoureteral reflux; CRF, chronic renal failure.

*Present age.

†Reference value: Ca, 2.18–2.53 mmol/l; P, 0.77–1.39 mmol/l in adults and 1.23–2.10 mmol/l in children; and intact PTH, 10–65 ng/l.

‡Degree of hearing loss: normal, ≤25 dB; mild, 26–40 dB; moderate, 41–55 dB; moderately severe, 56–70 dB; severe, 71–90 dB; and profound, >90 dB.¹³

§Age at death.

¶On treatment.

**Using hearing aids.

††Clinically suspected because of speech delay.

DNA was amplified by PCR for the six exons and flanking intron sequences with the nine sets of primers reported by Van Esch *et al*¹⁰ (University of Leuven, <http://www.kuleuven.ac.be/med/molonc/HDR.htm>). Subsequently, the PCR products were purified and subjected to cycle sequencing reaction with the same nine sets of primers and the following five new primers: 1S, ATACTGAGAGAG-GGAGAGAGAG; 1A, TCACCACTACCAACCTGGGT; 2S, GCTCATCCAGGTCTCCATT; 5S, CAAGCCTGTCTTCATAGTGATGACA; and 6S, GATTAACAGAC-CCCTGACTATGAAG. The primer positions are shown in fig 2B. Furthermore, to confirm mutations, PCR products with the mutations were digested with appropriate restriction enzymes.

Results

CYTOGENETIC STUDIES

The probands of families A-D had non-mosaic chromosomal aberrations involving 10p, as described in fig 1. The father of family A (case A/II.1) had a reciprocal 10p;18p translocation. The remaining subjects had normal karyotypes.

DELETION ANALYSIS OF DISTAL 10P

The results of FISH and microsatellite analyses in families A-D are summarised in table 3 and the deletion maps are shown in fig 2A. GATA3 was present in a single copy on the normal chromosome 10 alone and was absent from the abnormal chromosome 10 in the probands of families A-D (fig 3). The breakpoint resided between D10S1649 and D10S1720 in cases A/II.1 and B/II.4, between D10S585 and D10S547 in case C/II.1, and between D10S547 and D10S465 and between D10S189 and GATA3 in case D/II.1. The deleted chromosome 10 was determined to be of paternal origin in families A, C, and D, and of maternal origin in family B. In case A/II.1, GATA3 was detected on the normal chromosome 10 and on the derivative chromosome 18, confirming the reciprocal translocation. In the remaining subjects, GATA3 was shown to be present in two copies by FISH analysis.

MUTATIONAL ANALYSIS OF GATA3

The results are summarised in fig 2B. Case E/III.1 had a heterozygous missense mutation within the ZF1 domain at exon 4 (T823A, W275R), case F/II.2 had a heterozygous unusual mutation at exon 4 (900insAA plus 901insCCT or C901AACCCT) resulting in a premature stop at codon 357 with loss of the ZF2 domain, and case G/II.2 had a heterozygous nonsense mutation at exon 6 (C1099T, R367X). These mutations were absent in 100 alleles of normal subjects. The mutation in case E/III.1 should create a *DdeI* site and this was confirmed by *DdeI* digestion of the PCR product for exon 4. The mutation in case F/II.2 was detected in the affected family members and undetected in the

Table 2 The results of immunological evaluation in three patients with HDR syndrome

Patient (age at evaluation)	A/II.1 (1.0 y)	B/II.4 (3.5 y)	C/II.1 (2.7 y)
T cells (%)	72 (53.9–80.5)	80 (41.8–77.8)	73 (44.3–73.3)
B cells (%)	25 (3.6–22.6)	17 (1.7–16.8)	23 (2.2–20.2)
Serum immunoglobulin levels (mg/dl)			
IgG	995 (460–1220)	926 (560–1390)	1290 (530–1340)
IgA	32 (16–128)	79 (31–202)	172 (25–174)
IgM	65 (57–260)	72 (66–288)	223 (63–279)
Mitogen stimulation tests* (cpm, ³ H-thymidine uptake)			
PHA	25 383 (26 000–53 000†)	55 139 (26 000–53 000†)	50 038 (26 000–53 000†)
Con-A	26 816 (20 000–48 000†)	40 487 (20 000–48 000†)	46 802 (20 000–48 000†)
Lymphocyte surface markers (%)			
CD4 (%)	NE	38.8 (23.7–57.3)	30.0 (23.6–61.0)
CD8 (%)	NE	33.1 (12.3–32.9)	23.0 (8.8–30.8)

PHA, phytohaemagglutinin; Con-A, concanavalin A; CD, cellular (cluster) differentiation antigen; NE, not examined.

The values in parentheses except for those for mitogen stimulation tests represent age matched reference data.^{14,15}

*Final concentration of a mitogen is 20 µg/ml for PHA and 7 µg/ml for Con-A.

†Reference values for normal adults.

clinically normal family members by sequencing; furthermore, the mutation should destroy a *Mnl*I site and this was shown by *Mnl*I digestion of the PCR product for exon 4. The mutation of case G/II.2 was detected in the affected family member and undetected in the clinically normal family members by sequencing; furthermore, the mutation should create an *Akw*NI site and this was shown by *Akw*NI digestion of the PCR product for exon 6. By contrast, no mutations were identified in cases H/II.1 and cases I/III.1, I/II.8, and I/II.9.

In addition, a G/A polymorphism was found at the 119th nucleotide in the non-coding region of exon 2; the genotype was G/A in cases E/III.1 and G/II.2, A/A in cases F/II.2 and H/II.1, and G/G in case I/III.1. In family I, in which no mutation was identified, the clinically normal parents also had the G/G genotype. This variation was also detected in normal Japanese subjects with a frequency of 40% for G and 60% for A.

Discussion

In the present study, heterozygous GATA3 abnormalities were identified in seven of the nine families examined. Whole GATA3 dele-

tions were found in families A–D, and three novel intragenic mutations including two mutations leading to premature terminations were found in families E–G. In this regard, despite the absence in 100 alleles of normal subjects, the possibility that the T823A (W275R) substitution in case E/III.1 might be a rare polymorphism cannot be excluded formally, because functional studies have not been performed. However, the tryptophan at codon 275 is located within the ZF1 domain and is highly conserved in other GATA family members and in mouse GATA3 (GeneBank, <http://www2.ncbi.nlm.nih.gov/Genbank/>), suggesting the biological importance of this residue. Thus, the T823A (W275R) substitution is considered to be a true mutation rather than a rare polymorphism. The results, in conjunction with those reported by Van Esch *et al.*¹⁰ imply that HDR syndrome is primarily caused by haploinsufficiency of GATA3.

The triad of HDR syndrome was variably exhibited by patients with GATA3 haploinsufficiency. For example, cases B/II.4 and C/II.1 had severe manifestations of each feature of the triad, case A/II.1 had a combination of severe hearing defect, mild renal lesion, and transient

Table 3 The results of FISH and microsatellite analyses in families A–D

Locus/region* (distance from 10ptel)	Methods† (FISH probe)	Family A			Family B			Family C			Family D		
		F	P	M	F	P	M	F	P	M	F	P	M
10p telomere region	FISH (2189b6/cosmid)	–	–	–	–	–	–	–	–	–	+	+	+
D10S552 (14.33 cM)	FISH (809F09/YAC)	–	–	–	–	–	–	–	–	–	+	+	+
D10S552	MS	5	4‡	4	1	1‡	3, 4	1, 4	5‡	3, 5	1, 4	1	1, 2
D10S189 (19.00 cM)	MS	1, 3	3	1, 3	3, 4	3	3, 4	1, 4	1	1, 4	2, 3	1, 2§	1, 3
D10S1751 (20.75 cM)	MS	1, 3	1	1, 2	3, 5	5‡	2	1, 4	5‡	2, 5	1, 2	2	2
GATA3	FISH (554F11/BAC)	–	–	–	–	–	–	–	–	–	–	–	–
D10S1779 (22.78 cM)	FISH (42I17/BAC)	–	–	–	–	–	–	–	–	–	–	–	–
D10S1779	MS	1, 2	2	1, 2	3	3	3, 4	1, 2	2	1, 2	1, 3	3	3
D10S226 (27.19 cM)	FISH (182F10/BAC)	–	–	–	–	–	–	–	–	–	–	–	–
D10S226	MS	2, 4	2	2, 5	1, 2	1	1, 5	4, 5	5	3, 5	1, 4	5‡	2, 5
D10S1720 (27.19 cM)	FISH (249K20/PAC)	–	–	–	–	–	–	–	–	–	–	–	–
D10S1720	MS	2, 6	2	2, 3	1, 4	1	1	2, 4	3‡	2, 3	3, 5	3	1, 3
D10S1649 (27.19 cM)	MS	2, 4	3, 4§	3, 6	1, 6	3, 6§	3, 5	4, 6	7‡	3, 7	2, 3	1‡	1, 4
D10S465 (28.31 cM)	MS	2, 3	2, 3§	2, 3	2	2, 3§	3	1, 3	2‡	2, 3	2	3‡	3
D10S547 (29.15 cM)	MS	1, 2	2	2	2	2	2	2, 4	3‡	2, 3	2, 3	2, 3§	2, 3
D10S585 (30.00 cM)	FISH (765A04/YAC)	+	+	+	+	+	+	+	+	+	+	+	+
D10S585	MS	1	1, 3§	3, 4	5	4, 5§	4, 6	2	2, 6§	1, 6	4, 6	4, 6§	6
D10S223 (32.80 cM)	MS	1	1	1, 2	1, 2	1, 2§	1, 2	1	1	1	2, 3	1, 3§	1
D10S191 (37.90 cM)	MS	2, 3	2, 7§	3, 7	1, 2	1, 2§	1	2, 5	2, 4§	2, 4	2, 6	3, 6§	3, 7

F, father; P, proband; M, mother; FISH, fluorescence in situ hybridisation analysis; MS, microsatellite analysis.

*The locus order and the genetic distance from the 10p telomere are primarily based on the chromosome 10 linkage map of the Marshfield Clinic (<http://research.marshfieldclinic.org/genetics/>).

†Resources for FISH probes: Ning *et al.*¹⁶ Children's Hospital, Oakland Research Institute (<http://www.chori.org/bacpac/>), Incyte Genomics (<http://www.incyte.com/reagents/index.shtml>), and Foundation Jean Dausset CEPH (<http://www.cephb.fr/>).

Resources for MS primers: Genome Database (<http://www.gdb.org/>).

The (+) and (–) symbols for FISH analysis represent the presence or absence of the examined loci/region on the abnormal chromosome 10, respectively.

The alleles determined by MS analysis are arbitrarily indicated by Arabic numbers according to their size: ‡confirmed hemizygosity and §confirmed heterozygosity.

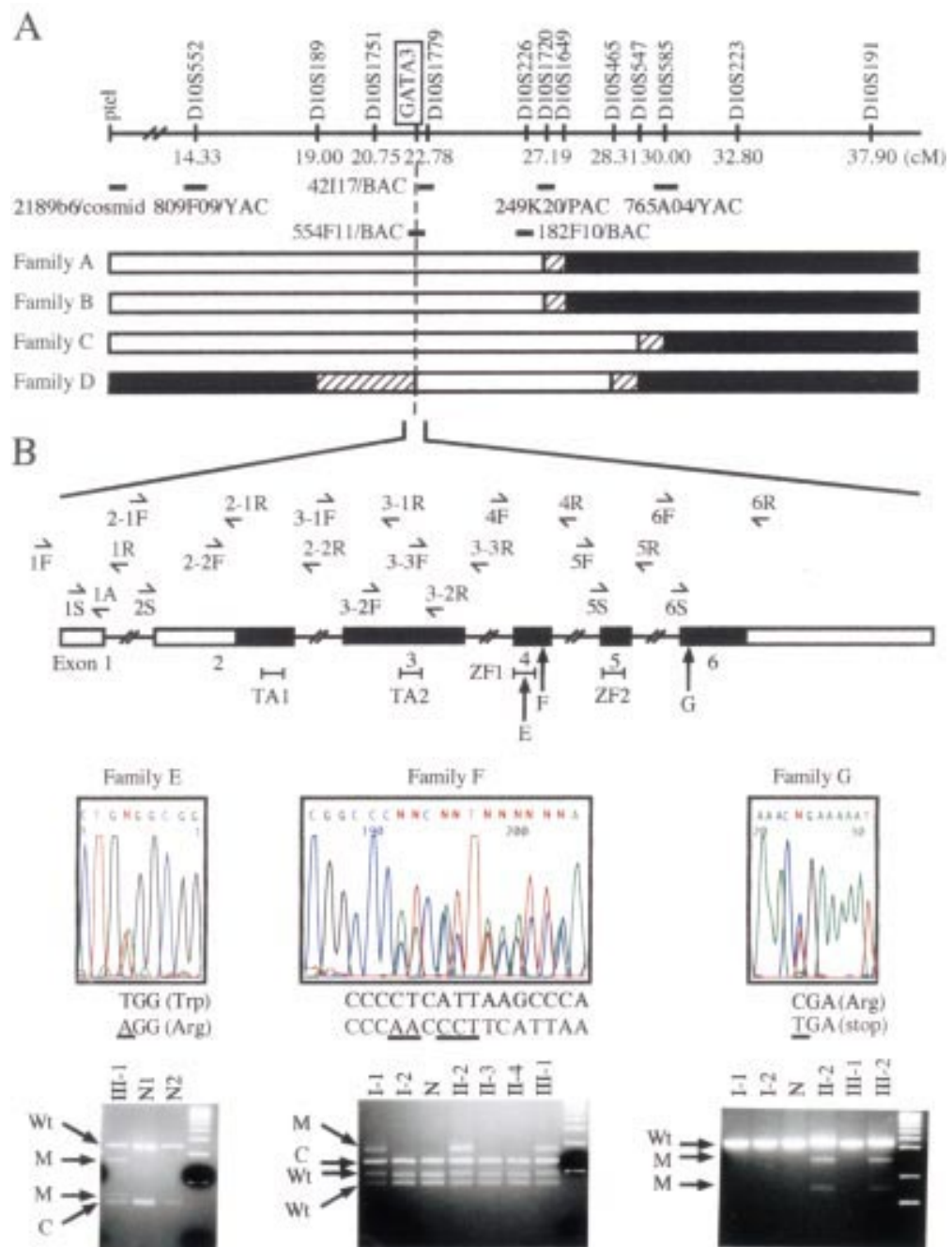


Figure 2 Molecular studies of the *GATA3* gene. (A) FISH and microsatellite analyses of 10p loci/regions around *GATA3*. The genetic map of *GATA3* (indicated by a square box) and 12 microsatellite loci is primarily based on the chromosome 10 linkage map of the Marshfield Clinic (<http://research.marshfieldclinic.org/genetics/>). The genetic distance from the 10p telomere is shown in cM (see also table 3). The FISH probes 2189b6/cosmid,¹⁶ 809F09/YAC, 554F11/BAC, 42I17/BAC, 182F10/BAC, 249K20/PAC, and 765A04/YAC contain 10p telomere region, D10S552, *GATA3*, D10S1779, D10S226, D10S1720, and D10S585, respectively. In families A–D, the white and black areas denote the monosomic and disomic regions, respectively, and the striped areas depict the dosage unknown regions where the breakpoints should exist. (B) Sequence analysis of *GATA3*. The *GATA3* gene consists of six exons and contains two transactivating domains (TA1 and TA2) and two zinc finger domains (ZF1 and ZF2). The black segments indicate the coding region. The primer positions are shown by arrows. Case E/III.1 is heterozygous for a missense mutation at exon 4 (T823A) which creates a DdeI site, and DdeI digestion of the PCR products has produced four fragments (237 bp, 165 bp, 72 bp, and 59 bp) in case E/III.1 and two fragments (237 bp and 59 bp) in normal controls (N1 and N2). Case F/II.2 is heterozygous for an unusual mutation at exon 4 (900insAA plus 901insCCT, or C901AACCCT) which destroys a MnlI site, and MnlI digestion of the PCR products has yielded four fragments (171 bp, 130 bp, 91 bp, and 75 bp) in affected family members (cases F/I.1, F/II.2, and F/III.1) and three fragments (130 bp, 91 bp, and 75 bp) in clinically normal family members (cases F/I.2, F/II.3, and F/II.4) and a normal control (N). Case G/II.2 is heterozygous for a nonsense mutation at exon 6 (C1099T) which creates an AlwNI site, and AlwNI digestion of the PCR product has produced three fragments (442 bp, 290 bp, and 152 bp) in affected members (cases G/II.2 and G/III.2) and a single fragment (442 bp) in clinically normal family members (cases G/I.1, G/I.2, and G/III.1) and a normal control (N). Wt: wild type allele specific band; M: mutant allele specific band; C: band common to wild type and mutant alleles.

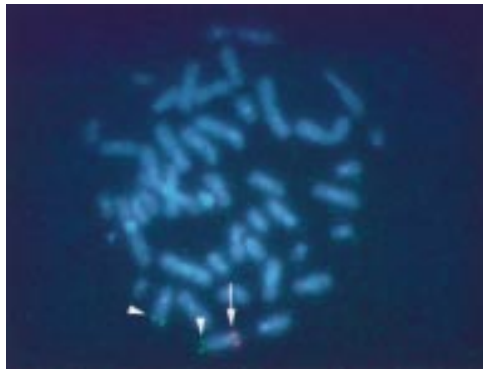


Figure 3 FISH analysis in case D/II.1. Only one single signal is detected for the GATA3 gene (arrow), whereas two signals are found for the 10q telomere (arrowheads).

hypocalcaemia, and cases F/III.1 and G/III.2 showed delayed verbal development as the sole possible HDR feature. Such phenotypic variability is not specific to GATA3 haploinsufficiency. It is known that haploinsufficiency of genes involved in human development frequently shows a wide range of penetrance and expressivity, depending on other genetic and environmental factors.¹⁷ Furthermore, although urinary anomalies were diverse, this would not be inconsistent with a single gene mutation. For example, mutations of the angiotensin type 2 receptor gene have been shown to result in variable patterns of urinary anomalies, because of delayed apoptosis of undifferentiated mesenchymal cells surrounding the Wolffian duct and ureter during renal and urinary tract development.¹⁸ Thus, clinical diversity in the HDR triad appears to be consistent with this syndrome being a developmental disorder. In addition, lack of appropriate investigations in several patients and probable phenotypic change with age could also account for phenotypic variability, because some features may be overlooked without pertinent examination and may remain undetected if not assessed at an appropriate age (for example, case E/II.5 first showed signs of hypoparathyroidism at 72 years of age).

Other clinical features were also observed in several patients with GATA3 haploinsufficiency. In this context, the repeated cerebral infarction in case D/II.1 might be related to GATA3 haploinsufficiency, because GATA3 is expressed in CNS.⁹ By contrast, pyloric stenosis in case B/II.4 and ventricular septal defect in case C/II.1 would be independent of GATA3 haploinsufficiency, because GATA3 is not expressed in stomach or heart. Similarly, non-specific features such as growth failure, mental retardation, and minor anomalies in the probands of families A-D would be the result of chromosomal imbalance rather than GATA3 haploinsufficiency, because such non-specific features have widely been observed in chromosomal abnormalities.¹⁹ Thus, with the possible exception of cerebral infarction, there appears to be no recognisable clinical features, other than the HDR triad, that are directly ascribable to GATA3 haploinsufficiency. In this regard, it may be worth pointing out that, despite GATA3 expression in thymus and T lymphocyte

lineages,^{5,9} immune related features were absent in all the patients, and immunological evaluations were grossly normal in three patients with whole GATA3 deletions. This suggests that a single copy of GATA3 is usually sufficient to maintain cellular immune function.

GATA3 abnormalities were not detected in families H and I. Van Esch *et al*¹⁰ have also failed to identify GATA3 abnormalities in two of five HDR families with no discernible cytogenetic abnormalities. In the present study, case H/II.1 had typical features of HDR syndrome, whereas case I/III.1 showed atypical features such as retinitis pigmentosa and severe growth failure, together with the HDR triad phenotype. Also in family I, clinical features in other family members and their segregation pattern also appear to be atypical of HDR syndrome. At this time, however, it remains to be clarified whether HDR syndrome is a genetically heterogeneous condition, or whether a mutation remains undetected in families H and I, because a partial deletion of the GATA3 gene might have been overlooked and the promoter region and the intron sequences have not been examined.

In summary, the present study provides further evidence that GATA3 haploinsufficiency is the major cause of HDR syndrome and indicates that this syndrome is associated with a wide phenotypic spectrum. Further studies in fully or partially affected patients will permit a better definition of the frequency of GATA3 abnormalities and the phenotypic spectrum of HDR syndrome.

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